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Effects of Axial Vibration on Needle Insertion into the Tail Veins of Rats and Subsequent Serial Blood Corticosterone Levels

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Blood collection is commonplace in biomedical research. Obtaining sufficient sample while minimizing animal stress requires significant skill and practice. Repeated needle punctures can cause discomfort and lead to variable release of stress hormones, potentially confounding analysis. We designed a handheld device to reduce the force necessary for needle insertion by using low-frequency, axial (forward and backward) micromotions (that is, vibration) delivered to the needle during venipuncture. Tests with cadaver rat-tail segments (n = 18) confirmed that peak insertion forces were reduced by 73% on average with needle vibration. A serial blood-sampling study was then conducted by using Sprague–Dawley rats divided into 2 groups based on needle condition used to cause bleeds: vibration on (n = 10) and vibration off (n = 9). On 3 days (1 wk apart), 3 tail-vein blood collections were performed in each subject at 1-h intervals. To evaluate associated stress levels, plasma corticosterone concentration was quantified by radioimmunoassay and behavior (that is, movement and vocalization) was scored by blinded review of blood-sampling videos. After the initial trial, average corticosterone was lower (46% difference), the mean intrasubject variance trended lower (72%), and behavioral indications of stress were rated lower for the vibration-on group compared with the vibration-off group. Adding controlled vibrations to needles during insertion may decrease the stress associated with blood sampling from rats—an important methodologic advance for investigators studying and assessing stress processes and a refinement over current blood sampling techniques.

Blood sampling is one of the more common practices in biomedical research involving animals, most often mice and rats. Several techniques and routes for obtaining blood samples exist.[23,52] Some routes require or benefit from anesthesia (for example, jugular, retroorbital), whereas others do not (for example, tail vein or artery, saphenous vein, facial vein). Sampling stress, induced by potentially painful needle punctures, physical restraint, and handling, can influence stress hormone levels to various degrees,[17,49] potentially confounding subsequent blood chemistry analysis. In addition, guidelines have been established for the frequency and volumes of blood that can be collected from an animal, usually determined by the animal's age and weight.[43] Repeated or serial sampling in a single animal is often difficult, especially for studies requiring multiple samples within a brief period of time, possibly because of tissue damage or stress-induced vasoconstriction. To overcome the effects of sampling stress and to ensure that sufficient blood volumes can be obtained, experimenters often rely on the collection of terminal blood samples, where the stress response can be avoided altogether.[49] When multiple time points are required, researchers typically use cross-sectional study designs using large numbers of animals, which are euthanized at different time points to obtain terminal blood samples.[10,22] This approach can be expensive, uses large numbers of animals, and limits the data that can be obtained, given that the data are derived from multiple animals rather than a single animal tracked over time.[22] The ability to follow individual animals over their lifespan is highly desirable. New minimally perturbing blood-sampling strategies are highly desirable, to enable serial blood sampling over an animal's lifespan for the execution of longitudinal studies that are not confounded by sampling stress.

The stress and discomfort associated with blood sampling can be influenced by several factors, including handling technique, restraint methods, anesthesia, collection volume, and invasiveness.[51] Because handling the animals during the procedure typically is unavoidable, the time involved should be minimized, and the restraint technique should be the least restrictive possible.[49] Stress can also be reduced through habituation by handling the animals in a highly similar way on a regular basis for a week or two before the actual blood-sampling procedures are performed. Even with minimization of the handling and restraint stress, it is common knowledge that the standard needle-puncture process is painful. In fact for humans, this painfulness is a key factor leading to noncompliance in the treatment of diabetes and other disorders that require needles to penetrate the skin.[3,9,42] The current study addresses the invasiveness component of sampling stress by evaluating a method designed to reduce the discomfort of needle insertion.

Research has shown that needle insertion pain varies inversely with the insertion force required to penetrate the skin.[14,19,21] Needle designs (for example, smaller diameter, sharper) and insertion strategies (for example, increased velocity) that reduce the penetration force and associated tissue deformation seem to reduce associated pain and discomfort.[28] Vibrating needles during insertion leads to reductions in both puncture and friction forces.[8,25,29,37,39,46,54] A similar phenomenon is used in nature by mosquitos, which vibrate their proboscis to penetrate the skin of their host.[11,13,31,39,45,54] The increased needle velocity from vibration decreases tissue deformation, energy absorbed,

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penetration force, and tissue damage.[35,36,50] Furthermore, mechanical vibration itself can produce an anesthetic effect.[17,41,47] The tissue vibration produced by the vibrating needle may stimulate nonnociceptive Aβ fibers, inhibit the perception of pain, and alleviate the sensation of pain at the spinal cord level.[17,27,34,37,40,41,47]

The objectives of the present study were to evaluate whether axial (backward and forward) micromotions (that is, vibration) of the puncturing needle decreased needle insertion forces and lead to less stressful tail-vein blood sampling in Sprague–Dawley rats that were sampled repeatedly on multiple days over several weeks.

Materials and Methods

Device details. Vibration was produced by a custom handheld device (Figure 1) that incorporated a voice-coil–style linear motor. The device used in this study was an early prototype of the commercially available GentleSharp device (Actuated Medical, Bellefonte, PA).[2] To create the vibration with the device, a sinusoidal alternating-current signal was applied to a coil, which created an alternating magnetic field and forcing a set of magnets to oscillate inside the handpiece housing. The set of magnets was fixed to a motor shaft, which had a Luer-lock adapter fixed at one end for the attachment of any Luer hub needle. In this study, 25-gauge, 1 in. hypodermic needles (no. 305125, PrecisionGlide, Becton Dickinson, Franklin Lakes, NJ) were used. Custom control electronics incorporating a microcontroller and power amplifier were developed to power the handpiece. The frequency of vibration was 150 Hz, and the free displacement amplitude in air was approximately 0.6 mm from peak to peak, verified with a linear variable differential transformer (CD375-100, Macro Sensors, Pennsauken, NJ).

Tests of Rat-tail insertion force. A set of experiments was performed by using cadaver rat-tail segments to compare the average penetration force of vibrated and nonvibrated needles. Tails from previously euthanized rats (n = 6) were obtained and stored in a freezer (less than 3 mo). Prior to testing, tails were thawed at room temperature and divided into 2- to 3-cm segments representing 3 different locations along the tail. At test time, the tail segments were placed in a low-friction support trough and fixed at one end to the tip of a force gauge (model FGV-1X, Nidec Shimpo America, Itasca, IL) by using cyanoacrylate. The analog output from the force gauge was wired to a data acquisition module (model NI-9205, National Instruments, Austin, TX), which was controlled by a custom LabVIEW program (National Instruments) that recorded the data for offline analysis (sampling rate, 20 samples per second). For each insertion, a new 25-gauge, 1 in. hypodermic needle was attached to the handpiece. As the operator held the handpiece at a 15° angle (approximately) to the skin surface, the needle was manually inserted into the rat tail. The force gauge registered the component of insertion force that was parallel to the main axis of the tail. Two insertions were performed bilaterally in each tail segment; in one insertion, the needle was vibrated, but it was not vibrated for the opposite side. Tests were performed in a total of 6 different tail segments (each from a different rat) for each of the 3 anatomic locations. Analysis of the force profiles focused on 2 parameters: the peak force (largest positive magnitude) recorded during needle insertion and the peak force (largest negative magnitude) recorded during removal. The insertion phase contains both tissue-puncturing forces and frictional forces between the needle and surrounding tissue, whereas the removal phase is dominated by frictional forces.

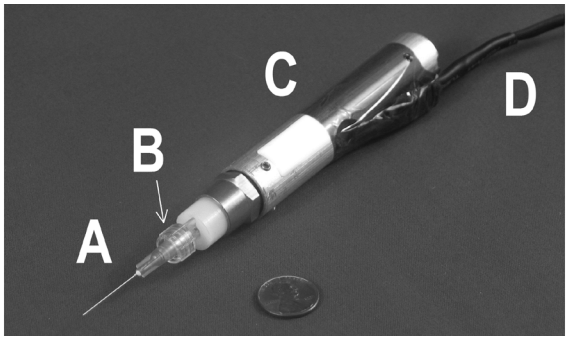

Figure 1. Prototype device for producing needle vibration. A, standard Luer hub needle; B, Luer attachment; C, handpiece housing; D, power cable.

Animals. The study population comprised 19 male Sprague–Dawley rats (age, 8 wk; weight, 250 to 275 g; Harlan Laboratories, Indianapolis, IN). Rats arrived at the animal facility 1 mo prior to the study and were housed in pairs or a trio (one cage only). For all but 2 of the dual-housed cages, one rat was assigned to each experiment group. Of the remaining cages, one had both subjects belonging to vibration-on group, and one had both subjects belonging to the vibration-off group. The trio cage housed 2 subjects belonging to the vibration-on group and the remaining rat to the vibration-off group. Rats were housed in a temperature- and humidity-controlled environment (22 ± 1°C; 45% to 55%), maintained on a 12:12-h light:dark cycle (lights on, 0700), and had unrestricted access to water and food (Rodent Diet 5001, LabDiet, St Louis, MO) throughout the study. Each rat was gently handled once daily during the 5 d prior to the first blood collection. Each handling session was approximately 3 min, during which each rat was picked up and allowed to walk around in the experimenter's hands. All procedures were reviewed and approved by the Pennsylvania State University IACUC and were performed in accordance with *The Guide for the Care and Use of Laboratory Animals*.[26]

Blood collections: serial sampling protocol. Rats were randomly assigned to one of 2 experimental groups: vibration-on (vibrated needle, n = 10) and vibration-off (nonvibrated needle, n = 9); no cross-over of animals occurred. Serial blood samples were completed in each rat over 3 wk, with a single collection day during each week, according to the experiment design (Figure 2).

On each sampling day, 3 blood collection trials were attempted at 1-h intervals in each subject with the vibration device (Figure 1) turned on or off, depending on group assignment. All collection trials occurred 2 to 6 h into the light cycle at the nadir of the diurnal corticosterone rhythm. Each cage was brought into the procedure room individually. For half of the cages, the rat belonging to the vibration-on group was sampled first, and for the other half the rat from the vibration-off group was sampled first. Prior to the collection trial, the rat-handling assistant submerged the tail of the subject in warm tap water (estimated 33 to 38 °C) for approximately 30 s. The subject was then gently placed on a cage top filter and lightly restrained (Figure 3). The experimenter attempted to puncture a lateral tail vein by using a new 25-gauge, 1-in. hypodermic needle to produce bleed with the device turned on or off, depending on the experimental group. Puncture sites for tail bleeds across the 3 trials of the collection day proceeded from distal to proximal along the tail. A maximum of 3 punctures were performed per collection trial. According to approved protocol, the trial was abandoned after 3 needle insertions, even when insufficient blood was obtained for the plasma corticosterone assay. After successful skin puncture,

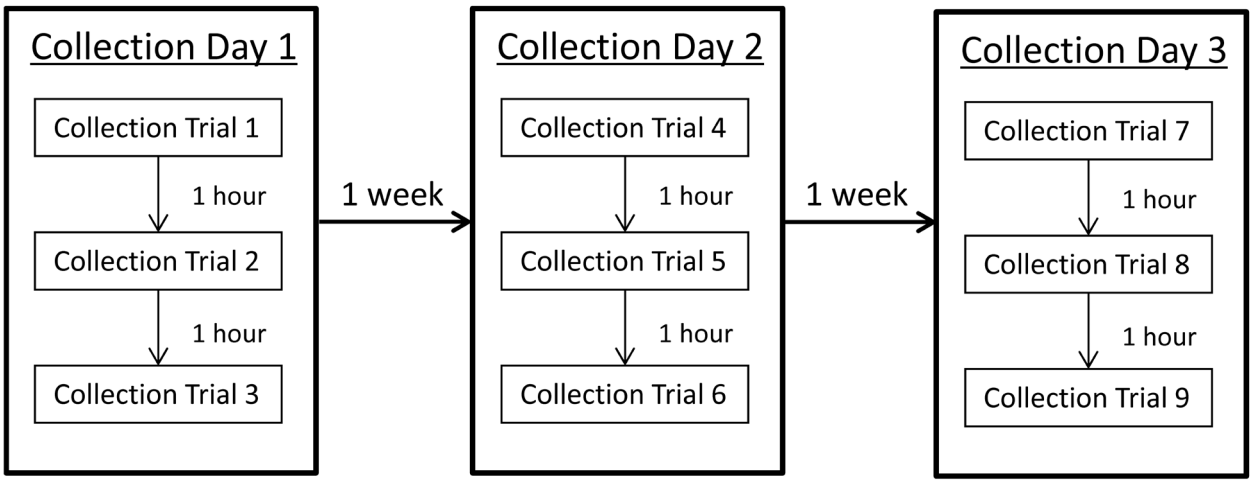

Figure 2. Experimental design of the rat tail-vein serial blood-sampling study.

blood was collected into lithium heparin minicapillary collection tubes (125 µL; catalog no. 07-6101, Safe-T-Fill, RAM Scientific, Yonkers, NY). After each collection trial, subjects were returned to their home cages. Samples were briefly centrifuged (3000 × *g*, 5 s) to evacuate all blood out of the capillary tube and into the collection vial. Samples were maintained at room temperature until all trials were complete for the collection day, after which the samples were centrifuged at 16,100 × *g* for 10 min, and plasma was aliquoted and frozen at –80 °C for later assessment of plasma corticosterone concentration.

Plasma corticosterone assays. Corticosterone in plasma samples was quantified by radioimmunoassay (catalog no. 07-120103, ImmuChem Double Antibody Corticosterone 125I RIA Kit, MP Biomedicals, Orangeburg, NY). The corticosterone antiserum reportedly cross-reacts with desoxycorticosterone (0.34%), testosterone (0.10%), and cortisol (0.05%). Serial dilutions of media samples over a 10-fold range produced a line parallel to the standard curve. After adjustment for dilution, the mean assayed value for the sample used for validation had a 4% coefficient of variation. The mean within-assay coefficient of variation for samples in all experiments was less than 6%. Assays were completed according to the manufacturer's instructions, except that reaction volumes were halved, and an additional 12 ng/mL standard point was included on the standard curve. The radioimmunoassay samples were evaluated on an automated gammacounter (model E5002, Cobra II Auto Gamma Counter, Packard, Meridan, CT). All sample tubes were measured in a single run on the same day.

Behavioral scoring. Digital videorecordings of all blood collection trials were obtained for offline analysis of the rats' behavioral response during sampling procedure. Three observers blinded to experimental group viewed the videos and provided independent assessments of behavioral responses by using a Likert scale from 0 to 5 to rate both movements and vocalizations. For movement, a score of 0 indicated no perceptible movement, whereas 5 indicated jumping or pronounced struggling occurred. For vocalization, a score of 0 indicated that no sound was heard, whereas 5 indicated that multiple, loud vocalizations were heard. Observer scores were then averaged to obtain mean movement and vocalization scores for each group for each collection day. For evaluating statistical significance, scores were analyzed separately for each observer.

Statistical analysis. ***Needle forces.*** Peak insertion and removal force data from the rat-tail testing are reported as mean ± 1 SD. Differences between vibrated and nonvibrated conditions for both peak insertion and removal forces were analyzed for significance by Kruskal–Wallis one-way ANOVA by using the R statistical computing language.[44]

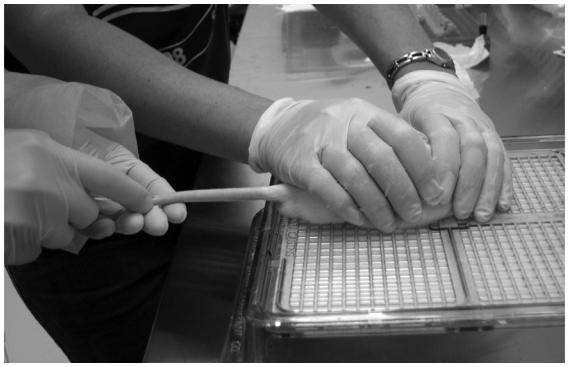

Figure 3. Photograph demonstrating the animal-handling and blood-sampling techniques used in the rat tail-vein serial blood-sampling study.

Corticosterone analysis. All descriptive statistics and related summary plots report untransformed corticosterone results as group means ± SEM unless otherwise noted. To assess the statistical significance of any intergroup differences, linear mixed effects analysis was conducted by using R[44] and the lme4 package.[7] The fixed effects included needle condition (vibrated compared with nonvibrated), collection day (day 1,2, or 3), trial type (1, first trial of the day; 2, second and third trials of the day), and the interaction of these 3 factors. The random effects included intercepts for subject as well as by-subject random slopes for the effect of collection day. To verify the selection of significant factors for the final statistical model, *P* values were obtained by likelihood ratio tests of the full model against simplified models with fewer fixed effects or interactions. Corticosterone values were strongly skewed right; therefore prior to linear mixed effects analysis, corticosterone values were transformed by natural logarithm to achieve a normal distribution and confirmed by visual inspection of histograms and QQ-plots of the log- transformed data. All standardized residuals were within 3 SD of the overall mean. Visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity or normality. The final linear mixed effects model fit the log(corticosterone) values at R^2 = 0.502. The R packages lsmeans[33] and multcomp[24] were used to perform posthoc analysis of the statistical significance of differences between groups by comparing the predicted marginal

means (least-squares means) obtained by linear mixed effects analysis. When multiple pairwise comparisons of the predicted marginal means were made, *P* values were adjusted by using either the Holm method or Tukey Honest Significant Difference to guard against increases in type I error rate. A *P* of 0.05 or less was considered statistically significant. To obtain a measure of effect size, the fixed effect of interest (needle condition, vibrated compared with nonvibrated) was divided by the residual standard error obtained from the linear mixed effects analysis.[30] As mentioned, descriptive statistics and figures are derived from untransformed data, unless otherwise noted, whereas all linear mixed effects analysis for evaluating statistical significance was performed on log-transformed data.

Behavioral scoring analysis. An index of interobserver agreement among the 3 observers was determined by using the Fleiss κ test. The 3 observers had slight agreement (κ = 0.166) for vocal scores and fair agreement (κ = 0.33) for movement. However, being that agreement between observers was not statistically significant, scores were analyzed separately for each observer, with overall results presented in summary form.

Results

Rat-tail peak insertion and removal forces. Vibration reduced both the peak insertion force and peak removal force. Differences between the 3 tail regions studied (Figure 4) were not statistically significant (data not shown), so all regions were pooled for subsequent analysis. The vibration of needles during insertion resulted in 73% lower peak insertion force (Kruskal–Wallis χ^2 = 26.27, df = 1, r = 0.85, $P < 5 \times 10^{-7}$; Figure 5) compared with that without vibration. In addition, the variation in peak insertion force was reduced, as indicated by comparative size of error bars (1 SD). In addition, vibration reduced the friction force between the needle and surrounding tissue, supported by 63% smaller peak removal force (Kruskal–Wallis χ^2 = 21.63, df = 1, r = 0.78 $P < 5 \times 10^{-6}$; Figure 5) compared with that without vibration.

Corticosterone analysis. The vast majority of rats in the vibration-off group had median corticosterone values that were higher than all of the median values for subjects in the vibration-on group (Figure 6 A). Overall, the vibration-off group also had a larger spread in corticosterone values (Figure 6 B); the difference in means of intrasubject variance was 72%. Intrasubject variances in corticosterone did not follow a normal distribution, so log-transformation was applied, and the intrasubject variances of the vibration-off and vibration-on groups were analyzed by 1-way ANOVA. The resulting analyses indicated a nonsignificant trend for lower intrasubject variance in the vibration-on group (P = 0.115, $F_{1,17}$ = 2.77). In addition, for every pair-housed cage containing a single subject from each group, the rat belonging to the vibration-on group had a lower mean corticosterone than did its vibration-off cage mate ($P = 7.81 \times 10^{-10}$; Figure 1 C)

A box-and-whisker plot summarizing the corticosterone results grouped by trial and needle condition is presented in Figure 7. For both groups, trial 1 yielded highly similar results for both groups, with very low variability among subjects. After this initial trial, the average corticosterone on subsequent trials was higher and more variable, even on the first trial of collection days 2 and 3 (trials 4 and 7). For every trial beyond trial 2, median values were lower in the vibration-on group compared with vibration-off group. Note that the number of observations for each trial was variable, due to the fact that the number of needle insertion attempts per trial was restricted to a maximum of 3; therefore, 45 (vibration off, 23; vibration-on, 22) of 171 total trials yielded insufficient blood sample to perform the corticosterone assay. The unbalanced nature of the dataset with multiple samplings from same subjects over time provided the primary justification for pursuing a linear mixed effects modeling approach for analyzing the significance of the corticosterone results and the effect of needle vibration.

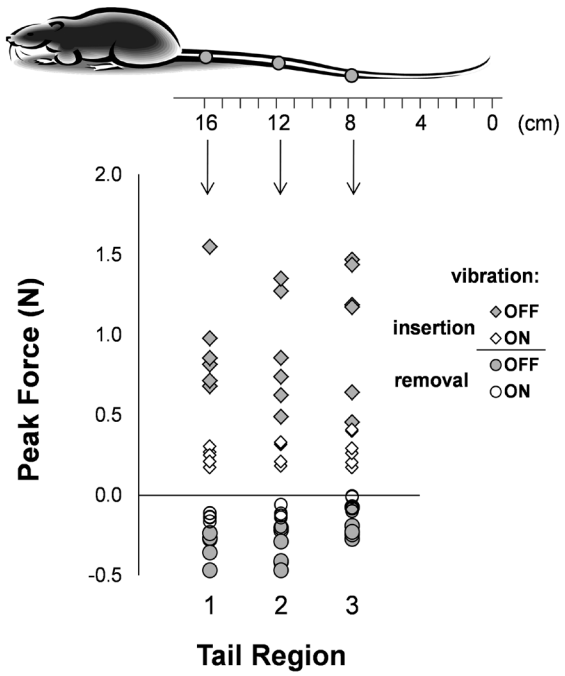

Figure 4. Needle vibration reduces the peak (maximal magnitude) insertion force and the peak removal force required for the insertion of 25-gauge needle into cadaver rat tails. Plot shows peak insertion force when vibration is off (gray diamonds) and on (white filled diamonds) and peak removal force (vibration off, gray circles; vibration on, white circles) for 3 different rat-tail regions (n = 6 tails tested). Vibration also reduced the variability in the peak insertion and removal forces.

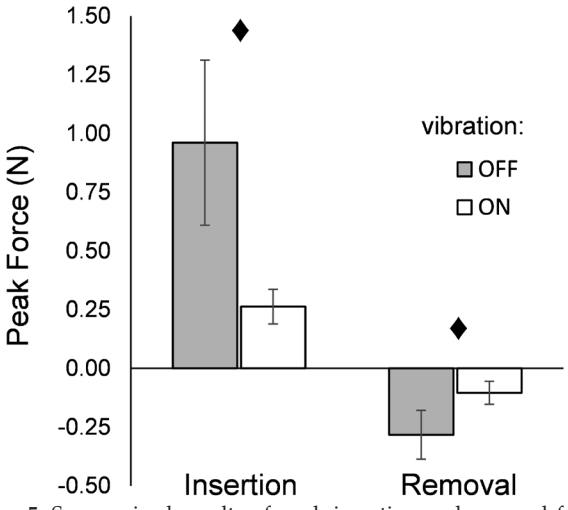

Figure 5. Summarized results of peak insertion and removal forces (combining all anatomic locations) for the rat-tail insertion experiment. With vibration on during puncture (white bars), the mean peak positive force during the insertion is reduced by 72.6% compared that when the vibration was off (gray bars). Likewise, vibration reduced the peak negative force during removal by 63.0%. ♦, $P < 1 \times 10^{-6}$, Kruskal–Wallis 1-way ANOVA, n = 18 insertion trials by using 3 tail segments from each of 6 rats; error bars, 1 SD.

The average corticosterone values for the type 1 trial (1st trial of the day) on collection day 2 were greater than those from the type 1 trial on collection day 1 for both groups (Figure 8). The differences were verified to be significant (vibration-off

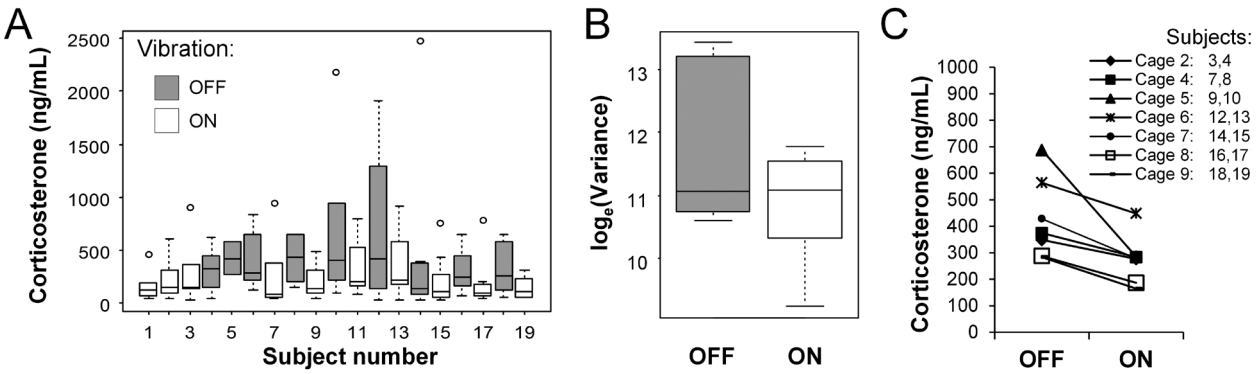

Figure 6. Summary of individual corticosterone data from the rat tail-vein blood-sampling study. (A) Box-and-whisker plot summarizing results from each subject in both groups, with gray indicating vibration off (nonvibrated needle) and white indicating needle on. (B) Box-and-whisker plot comparing mean intrasubject variability (log-transformation applied to achieve normality) for vibration-off (gray) and vibration-on (white) groups. There was a moderate trend toward lower intrasubject variability (% difference, 72%) in the vibration-on group compared with the vibration-off group; 1-way ANOVA of log-transformed intrasubject variability values: $F_{1,17} = 2.767$, $\eta^2 = 0.14$, $P = 0.115$. (C) Comparison of means of corticosterone values of cagemate pairs in the 2 groups.

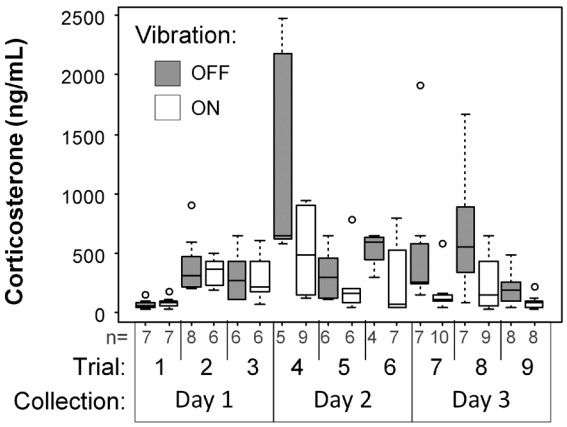

Figure 7. Box-and-whisker plot of corticosterone results for each individual trial from the rat tail-vein blood-sampling study, separated by experimental group (gray, vibration off; white, vibration on). The total number of observations for each trial, separated by group, is provided in the top row of the *x*-axis label. Because each trial was limited to 3 needle-puncture attempts, sufficient blood to perform corticosterone analysis typically was not available for every subject for a given trial.

group, $P < 0.0001$; vibration-on group, $P < 0.005$; by comparing least-squares means obtained by linear mixed effects analysis of log(corticosterone) with Tukey adjustment). The corticosterone concentrations from type 1 collections on collection day 3 compared with day 1 remained significantly higher for the vibration-off group ($P < 0.005$) but not for the vibration-on group ($P = 0.85$). Furthermore, the only sets of trials in the vibration-on group for which the corticosterone concentration varied significantly from the initial baseline (day 1, type 1 trial) was the type 1 trial on collection day 2 (previously mentioned) and the set of type 2 trials (2nd and 3rd trials of the day) on day 1. In contrast, for the vibration-off group, corticosterone concentrations from all sets of trials (both type 1 and type 2 trials) from any collection day were significantly higher ($P < 0.05$) than the vibration-off group starting baseline (day 1, type 1 trial). Tukey adjusted *P* values from pairwise comparisons of least-square means for the effect of different needle conditions (vibrated compared with nonvibrated), separated by trial type and collection day, were as follows: day 1, $P = 0.953$ and $P = 0.999$ (type 1 and type 2 trials, respectively); day 2: $P = 0.069$ and $P = 0.143$; and Day 3: $P = 0.041$ and $P = 0.051$. Although most *P* values were not significant when the individual type 1 and type 2 trials were analyzed separately, the trends were strong or significant between the vibration-off and vibration-on groups on collection days 2 and 3 for both type 1 and type 2 trials. When the entire dataset was evaluated, corticosterone values did not differ between type 1 and type 2 trials (linear mixed effects analysis, df = 84.3, t = 0.466, $P = 0.6422$), even though the effect of trial type was strong on day 1.

Pooling the data for type 1 and type 2 trials revealed significant differences in the mean corticosterone level between the vibration-on and vibration-off groups on collection days 2 and 3, with vibration-on group having 49% and 65% lower mean corticosterone, respectively (Figure 9 A). The differences between the least-squares means for the vibration-off and vibration-on group were statistically significant for days 2 and 3 ($P < 0.01$ for both; linear mixed effects analysis of log(corticosterone), df = 17, t = 3.332 and 3.451, respectively). When all trials were considered together, the rats in the vibration-on group, which had all collections performed with needle vibrating during insertion, had 46% lower corticosterone concentrations on average (Figure 9 B; comparison of the least squares means derived from linear mixed effects analysis of log(corticosterone): $P < 0.005$, df = 18.54, t = 3.755). In addition, a measure of effect size was obtained by dividing the fixed effects by the SD of the residual.[30] The effect size of the needle vibration was determined to be –0.8201.

Behavioral scoring. Consistent with the corticosterone results, the rats in the vibration-on group had lower average vocalization and movement scores on each weekly collection day (Figure 10). No observer saw a significant difference between needle conditions (vibration on and vibration off) for either vocal scores or movement scores on collection day 1. However, each observer noted a significant difference between the vibration-on and vibration-off conditions in the movement scores on collection day 2 (Kruskal–Wallis $\chi^2 = 9.69, 4.55, 4.90$, df = 1, $P < 0.05$). Two observers saw a significant difference between the vibration-on and vibration-off conditions for vocal scores on collection day 2 (Kruskal–Wallis $\chi^2 = 10.33, 8.56$, df = 1, $P < 0.005$); 2 observers saw a significant difference between vibration-on and vibration-off for movement scores on collection day 3 (Kruskal–Wallis $\chi^2 = 4.14, 6.57$, df = 1, $P < 0.05$), and 2 observers saw a significant difference between vibration-on and vibration-off for vocal scores during week 3 (Kruskal–Wallis $\chi^2 = 5.48, 9.43$, df = 1, $P < 0.05$).

Discussion

In this study, a handheld vibrating device, an early prototype of the GentleSharp system[2] which was designed to reduce needle insertion force, was evaluated for its ability to lower the overall stress induced by blood sampling procedures in rats.

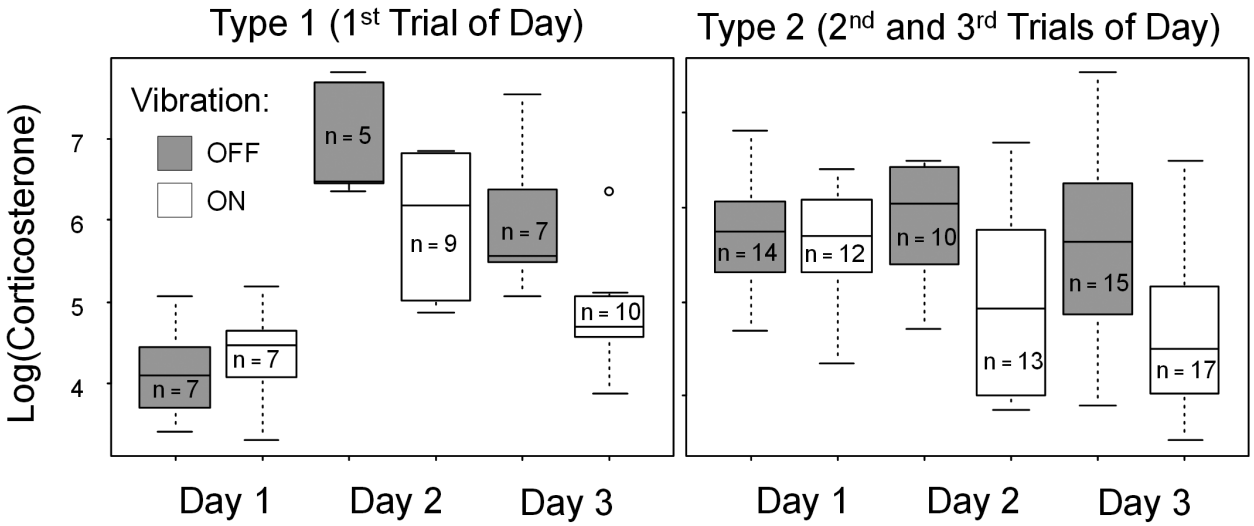

Figure 8. Box-and-whisker plot of log (corticosterone) results for type 1 trials (that is, the 1st trial of each collection day) and type 2 trials (that is, including both the 2nd and 3rd trials of each collection day), separated by experimental group (gray, vibration off; white, vibration on). Total observations for each group is provided inside the box. See the text for evaluations of the statistical significance of differences between predicted marginal means for each group.

The vibration of needles during insertion reduced the peak insertion force in cadaver rat tails by an average 73% and the peak removal force by 63% (Figure 5). We hypothesized that the force reduction provided by the vibrating needle would decrease the stress due to serial blood sampling, as reflected in the plasma corticosterone level. This hypothesis was supported by the fact that corticosterone was lower on average (46%) in rats in the vibration-on group, in which all collection trials used a vibrated needle to produce the puncture, as compared with the vibration-off group, in which the needles were not vibrated. The effect size (that is, fixed effect / SD of residual) of needle vibration was determined to be 0.8201. In addition, behavioral indicators of stress (that is, movement, vocalization) were lower for the vibration-on group than the vibration-off rats.

The mechanical effects of the vibrating needle on insertion force can partly be explained by the viscoelastic properties of the biologic tissue and can be understood through a modified nonlinear Kelvin model that captures the force-deformation response of soft tissue.[35,36] Because the internal tissue deformation for viscoelastic bodies is dependent on velocity,[4] increasing the needle insertion speed results in less tissue deformation. The reduced tissue deformation prior to crack extension increases the rate at which energy is released from the crack and ultimately reduces the force of rupture.[4,35] In addition to reducing the forces associated with cutting into tissue, research has shown that needle vibration during insertion reduces the frictional forces between the needle and surrounding tissues.[29] The insertion force results that we obtained in the current study confirm that the vibration of the needle during insertion reduced both the puncture and frictional forces.

The dynamics of the corticosterone response observed during collection day 1 were consistent with reports from other studies that evaluated corticosterone differences under repeated blood sampling over short time intervals.[20,48] One study that obtained tail vein blood samples at relative times of 0, 20, 60, and 120 min during the nadir and peak of the diurnal corticosterone rhythm found a significant increase in corticosterone for the 20- and 60-min time points, which presumably reflected the corticosterone response to the first blood-collection time point.[20] The starting baseline corticosterone levels were comparable for the previous[20] and current studies, but the shift in corticosterone levels from trial 1 to trials 2 and 3 was larger for the present study than the previous one. A possible explanation may be differences in preacclimation protocol. The rats in the present study were only gently handled, without fully going through collection procedure steps, including tail warming, temporary mild restraint, and exposure to the novel environment of the collection room. Larger and more variable corticosterone increases emerged in early repeated samples (15 and 30 min after the initial sample) from jugular-cannulated rats.[48]

The corticosterone dynamics of collection day 1 were as we expected, with the first trial being low (representing baseline) and the following 2 trials being elevated due to the corticosterone response triggered by the first trial. Interesting, this same pattern is not clearly repeated for collection day 2 or 3 for either group. Instead, it appears that the type 1 trials on collection days 2 and 3 do not reflect true baselines for either group. Overall, it is clear that the vibration-on and vibration-off groups had different corticosterone dynamics on collection days 2 and 3 compared with day 1, particularly the vibration-off group, which never returned to baseline values. Because the effect of trial type appeared to vary with collection day, this interaction term was included in the linear mixed effects model and verified to be significant. One possible explanation for why the type 1 trials may not represent true baseline on days 2 and 3 is that the precollection procedures (for example, room entry, cage transport, exposure to new environment) triggered an early corticosterone response. Because the vibration-on rats exhibited lower corticosterone levels in type 1 trials on collection days 1 and 2, the precollection stimulus might have elicited a stronger corticosterone response in the vibration-off group due to differences in the stress associated with the collection experience of the prior collection day. Furthermore, the vibration-on and vibration-off groups might have habituated to the repeated blood collections at different rates. The degree of the stressor has been shown to affect the dynamics of habituation (or sensitization) to stressors.[6] Finally, because the differences between vibration-on and vibration-off groups were significant only on collection days 2 and 3, the vibrating needle approach may not provide much benefit in reducing the corticosterone response when sampling is infrequent. However, that does not necessarily mean that the rats experience different degrees of pain depending on the needle

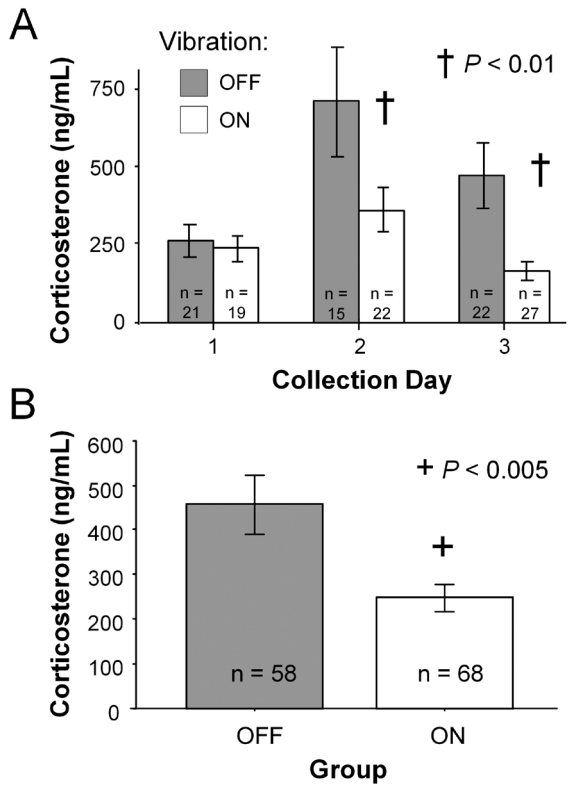

Figure 9. Averaged corticosterone results. (A) Weekly average comparison. Each bar represents the average of all collection trials that yielded sufficient blood for corticosterone analysis from each group, separated by weekly collection day (the number of observations for each collection day is provided). Linear mixed effects analysis of log-transformed corticosterone values with least-square means to evaluate contrasts of predicted marginal means reveals that mean corticosterone was significantly different on weekly collection days 2 and 3 (df = 17; week 2: t = 3.33, $P < 0.01$; week 3: t = 3.45, $P < 0.01$; P values adjusted by Holm method). (B) Overall average corticosterone comparison. Each bar represents the average of all collection trials that yielded sufficient blood for corticosterone assay from each group (vibration on, $n = 68$ samples from 10 rats; vibration off, $n = 58$ samples from 9 rats). The difference in means was 45.8% ($P < 0.005$, contrast of predicted marginal means computed with linear mixed model with log-transformed corticosterone values; df = 18.5, t ratio = 3.76, effect size [fixed effect / SD of residuals] = –0.82). Error bars, SEM.

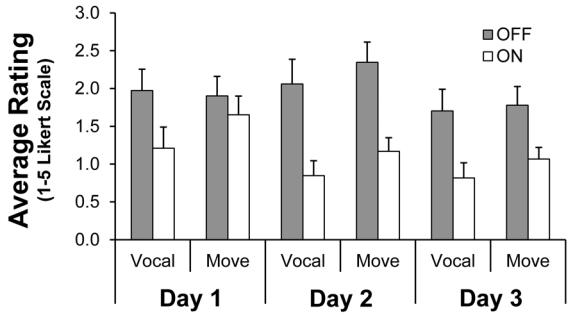

Figure 10. Average ratings for animal movement and vocalization during serial blood sampling from rat tail veins for the vibration-on group (gray bars) compared with the vibration-off group (black bars). Blinded observers scored videos of each blood collection trial with regard to animal vocalization (vocal) and movement (move) on a scale of 1 to 5; all reviewer scores were averaged to obtain a mean rating per trial. Each bar represents the average of mean ratings of all trials for the given week (3 trials per rat per weekly collection day). Error bars, SEM; see text for statistical analysis of observer scores individually by collection day.

condition. Instead, the differences corticosterone level might only be observed after the subjects became more acclimated to the overall procedure, such that the needle effect becomes more prominent relative to the handling effects. Future studies with improved and longer preacclimation procedures that better simulate the blood-collection process might reveal earlier differences in the corticosterone concentration.

Corticosterone is a notoriously 'noisy' measurement and can be influenced dramatically by a wide range of factors, many of which might appear relatively benign and a part of routine animal care, including cage changes, weighing, and moving subjects from one room to another.[5,18,38] Factors contributing to the lack of significance when trials were analyzed separately may be the variability among subjects, missing data, and the relatively small sample sizes. The average corticosterone level was lowest and showed the least intersubject variability for both groups at the outset of the study, prior to exposure of the rats to the blood-sampling process. After the initial trial, the rats from both groups experienced significant elevations in corticosterone concentration as well as intersubject variability, but the increases were higher in the vibration-off group. The mean and SEM of corticosterone concentrations observed in our study are comparable with results reported elsewhere.[6,20,32,48] In addition to corticosterone being influenced by many routine factors that are difficult to control, intrasubject variability corticosterone levels can be influenced by natural modulations in the basal corticosterone concentration. A high-frequency, automated blood sampling study showed that basal corticosterone is not constant during the nadir of the circadian rhythm but is pulsatile, with pulse heights of 75 ng/mL on average and with pulses occurring every approximately 45 min.[53] Furthermore, the same study showed that the corticosterone response to a mild stressor (white noise) depended on whether the stressor occurred during an increasing or decreasing phase of one of the basal corticosterone pulses.[53]

To avoid excessive handling of test subjects, some researchers place semichronic catheters, which are tethered to animals, and use specialized instruments to obtain blood samples automatically. This solution has its own drawbacks, given that animals must be transferred to a new cage and be singly housed throughout the collection period, both of which can be stressors on their own.[15,18,38] In addition, jugular cannulation has been associated with high failure rates after 1 wk, thus limiting long-term studies, and cannulation itself is a stressor because it limits the subject's mobility and activity levels.[12] Furthermore, cannulation is inefficient for studies involving large numbers of animals, and automated blood sampling itself is not fully immune from elevating stress hormone levels.[1] In the current study, handling stress remained and likely contributed to increased stress in both vibration-on and vibration-off groups. However the response was significantly higher in the vibration-off group, suggesting that simply modifying the venipuncture conditions by incorporating a vibrating needle can provide some reduction in the stressfulness of blood sampling.

The blood-collection method using the vibrating device described in this study addresses 2 of the '3 Rs' principals, refinement and reduction. In regard to refinement, rats in the vibrated needle group had reduced corticosterone levels. Moreover, rats in the vibration-on group exhibited decreased adverse behavioral indicators (that is, reduced animal movement and vocalization) of stress as compared with rats in the vibration-off group. Performing blood collections with the aid of the vibrating device refines the technique of tail sampling by improving on current methods, such as tail nicking or clipping for blood collection.

Regarding reduction, needle vibration reduced the levels of the stress hormone corticosterone and decreased the intraindividual variability in corticosterone levels throughout the study in the rats. The resulting improved reproducibility might allow for a reduction in the number of animals necessary in future studies. Indeed, this benefit would save researchers the cost of additional animals and would decrease sample-processing time. Moreover, by having the ability to serially sample the same animal, researchers can track an individual animal over time, thus allowing more complete data collection.

In summary, blood sampling is a common practice in biomedical research involving mice and rats and is often a required component of experimental design. Painful needle puncture can cause discomfort in research subjects and increase stress hormone levels, potentially confounding blood chemistry analysis and triggering vasoconstriction, ultimately making serial blood sampling difficult. Vibrating needles during insertion reduces the force required to penetrate tissues and may lead to less stressful venipuncture procedures in research subjects. Future studies might evaluate the expansion of the approach to other species and sampling locations and assess the influences of age and disease. The current technology is highly compatible with low-volume sampling with collection by capillary tubes (appropriate for research obtaining pharmacokinetic and pharmacodynamic data). Additional advances in this technology will allow for increased volumes of blood to be collected from larger animals and for injection capability. In conclusion, the current study may represent a particularly important methodologic advance for investigators interested in studying and assessing behavioral and physiologic stress processes and may serve to improve animal wellbeing.

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